JOURNAL OF BACTERIOLOGY

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0021-9193/84/100266-07$02.00/0

Vol. 160, No. 1

Aerobactin Genes in *Shigella* spp.

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Received 18 June 1984/Accepted 28 July 1984

Aerobactin, a hydroxamate iron transport compound, is synthesized by some, but not all, *Shigella* species. Conjugation and hybridization studies indicated that the genes for the synthesis and transport of aerobactin are linked and are found on the chromosome of *Shigella flexneri*, *S. boydii*, and *S. sonnei*. The genes were not found in *S. dysenteriae*. A number of aerobactin synthesis mutants and transport mutants have been isolated. The most common mutations are deletions of the biosynthesis or biosynthesis and transport genes. The *Shigella* aerobactin genes share considerable homology with the *E. coli* ColV aerobactin genes. On the ColV plasmid and in the *Shigella* chromosome, the aerobactin genes are associated with a repetitive sequence which has been identified as IS*1*.

Shigella species synthesize both phenolate and hydroxamate siderophores for acquisition of iron. *Shigella sonnei* utilizes enterobactin (enterochelin) (25), a phenolate siderophore common to many enteric species (19), and some strains produce an additional hydroxamate siderophore (25). Similarly, *S. boydii* has been reported to produce both phenolate and hydroxamate siderophores (25). *S. flexneri* strains normally synthesize only aerobactin (22), a secondary hydroxamate originally isolated from cultures of *Aerobacter aerogenes* (9) and more recently from *Escherichia coli* ColV strains (29). Rare isolates and laboratory variants of *S. flexneri* produce enterobactin in addition to aerobactin (23).

The genetics of iron transport in enteric bacteria has been studied primarily in *E. coli* and *Salmonella typhimurium*. The genes for enterobactin synthesis and transport are linked and are carried on the chromosome in both species (12–14, 26). Aerobactin genes, however, are encoded by the ColV plasmid in *E. coli* (29) and by a large plasmid in *A. aerogenes* (18). In other species, the location of the aerobactin genes is unclear.

Since differences were observed in the types of iron transport systems expressed by different *Shigella* species (22, 23, 25) and even by members of the same species, it was of interest to examine the genetics of siderophore synthesis and transport in *Shigella* spp. and to compare it to the ColV system.

MATERIALS AND METHODS

Strains and media. Bacterial strains, plasmids, a phage, and their sources are listed in Table 1. All other *Shigella* isolates were provided by Gloria Pierce, Texas Department of Health, Austin, Texas. Plasmid pKLS1 was a spontaneous deletion of pABN1. This plasmid was isolated by selecting ampicillin-resistant, cloacin-resistant mutants of pABN1. Ampicillin selects for maintenance of the pABN1 vector, whereas cloacin selects for loss of the 74K aerobactin receptor protein (3). This plasmid contains 1.7 kilobases (kb) of the original 16.3 kb of ColV sequences as determined by restriction enzyme digestion and hybridization with pABN1. The subclone pKLS10 was constructed by *Bgl*II restriction enzyme digestion and ligation of electroeluted fragments of pABN1. Both plasmids have the original vector, pPlac, from pABN1. Bacterial stocks were maintained frozen at −70°C in tryptic soy broth (Difco Laboratories) with 20% glycerol. Luria broth (15) was used for growth of *Shigella* strains. Low-iron Tris-buffered medium without added iron was used as described previously (22, 23) to determine siderophore production.

Siderophore assays. Strains were grown through two passages of Tris medium to induce siderophore synthesis. Phenolates were assayed by the colorimetric assay of Arnow (1), and hydroxamates were assayed by the ferric perchlorate assay of Atkin et al. (2) or the more sensitive Csaky test (6). Transport of siderophores was determined by a bioassay (22, 23) in which the ability of a siderophore to stimulate growth of bacteria in low-iron agar medium was determined. Siderophores were isolated by extraction with organic solvents and chromatography and were compared with aerobactin or enterobactin by thin-layer chromatography as previously described (22, 23).

Southern hybridization. Chromosomal DNA was prepared by the procedure of Marmur (17). *Shigella* plasmid DNA was isolated by the method of Hansen and Olsen (10), a procedure which allows isolation of large plasmids. Cleared lysates of sodium dodecyl sulfate-lysed cells (15) were the source of other plasmids. DNA was cut with restriction endonucleases (New England Biolabs) and electrophoresed through 0.9% agarose gels. DNA fragments were transferred to nitrocellulose (Schleicher & Schuell, Inc.) by the method of Southern (28) and were hybridized by that of Maniatis et al. (15). Hybridization probes were prepared by restriction enzyme digestion of plasmid or phage DNA and separation of insert fragments from vector on agarose gels. It was necessary to eliminate vector sequences since some of the small *Shigella* plasmids which frequently contaminate chromosomal DNA preparations were found to have homology with ColE1 vectors. DNA fragments were electroeluted into wells in the agarose filled with 50% glycerol in electrophoresis buffer. Eluted fragments were labeled with [^{32}P]dCTP (New England Nuclear Corp.) by nick translation (16). Only labeled fragments which did not show significant hybridization to vector DNA were used. Filters were hybridized at 68°C and washed at 68°C with 0.1 × SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for stringent hybridization conditions (15). The SSC concentration was increased to 1× when less stringent conditions were desired.

Conjugation. Overnight cultures of the donor (*S. flexneri* 256) and recipient (SA255) were diluted in Luria broth and

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TABLE 1. Bacterial strains, plasmids, and phage

Strain[a]	Source/Reference
Bacteria	
S. flexneri	
SA100 Hds$^+$ Hdu$^+$	23
SA201 Hds$^-$	23
SA255 Hds$^-$ Hdu$^-$	23
SA845 (F$^+$/tet^r)	This study
256 (Hfr)	R. Neill, Walter Reed Army Institute for Research (8)
S. dysenteriae 4576	Texas Department of Health
S. sonnei 1245	Texas Department of Health
S. boydii 1392	Texas Department of Health
Phage	
VAλ3 (IS*1*)	7
Plasmids	
ColV-K30	J. H. Crosa (29)
pABN1	4
pKLS10	Subclone of pABN1
pKLS1	Deletion plasmid derived from pABN1

[a] Hds, Aerobactin synthesis; Hdu, aerobactin utilization.

grown to mid-log phase. Equal volumes (1 ml) were mixed in a 125-ml flask and were incubated at 37°C for 2 h. The mixture was diluted and plated on Luria broth agar containing an antibiotic to select again the donor and 25 μg of deferrated ethylenediamine-di-(*o*-hydroxyphenylacetic acid) (EDDA; Sigma Chemical Co.) per ml (27). The iron chelator EDDA inhibits growth of cells which lack a siderophore-mediated iron transport system.

TABLE 2. Synthesis and utilization of siderophores by *Shigella* species

Species	Aerobactin		Enterobactin	
	Synthesis[a]	Utilization[b]	Synthesis[c]	Utilization[b]
S. boydii 1392	+ (0.08)	+	−	−
S. dysenteriae 4576	−	−	+ (0.16)	+
S. flexneri SA100	+ (0.07)	+	−	−
S. sonnei 1245	−	+	+ (0.27)	+

[a] Aerobactin synthesis determined by ferric perchlorate (2) and Csaky (6) assays and comparison of the isolated siderophore to aerobactin on thin-layer chromatography. Numbers in parentheses show the absorbance at 500 nm (by ferric perchlorate assay) of T medium culture supernatant.

[b] Determined by bioassay.

[c] Enterobactin synthesis determined by Arnow (1) assay and comparison of isolated phenolate to enterobactin on thin-layer chromatography. Numbers in parentheses show the absorbance at 515 nm (by Arnow assay) of T medium culture supernatant.

RESULTS

Strains of *Shigella* spp. were examined to determine the presence and location of the aerobactin genes. Initially, strains were grown in low-iron medium and tested for the ability to synthesize and utilize aerobactin and enterobactin. Results of representative strains of each species are shown in Table 2. In all, 2 strains of *S. dysenteriae*, 10 strains of *S. sonnei*, and 3 strains of *S. boydii* were tested. One hundred strains of *S. flexneri* had been assayed previously (23), and four additional strains were included in this study. No evidence for the secretion of hydroxamates by *S. dysenteriae* or *S. sonnei* was found. The ferric perchlorate (2) and Csaky (6) assays were negative, and no functional aerobactin was detected by bioassay. Both species were found to secrete and utilize enterobactin. Although the *S. sonnei* strains tested did not synthesize aerobactin, they were able to transport the siderophore and utilize it for growth in low-

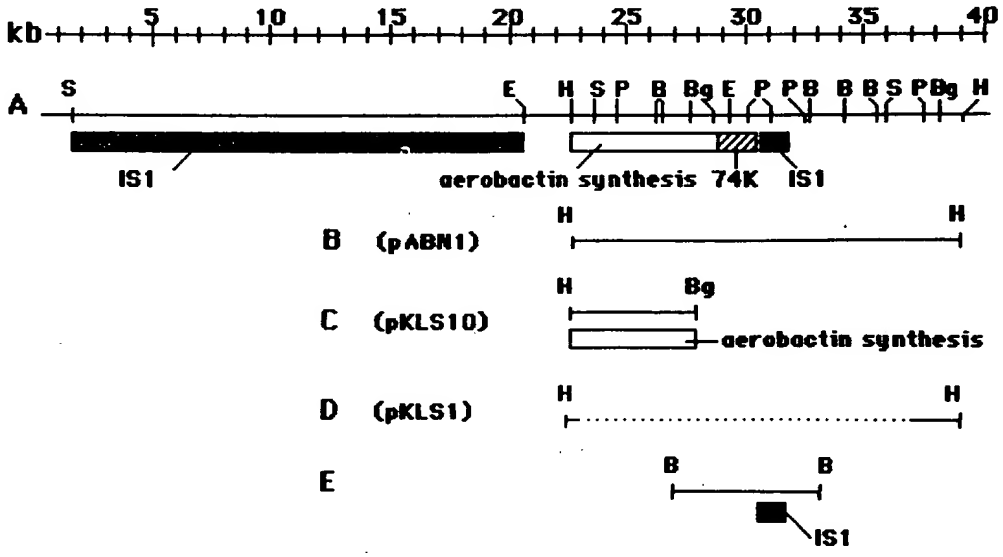

FIG. 1. Partial restriction map of ColV-K30 and hybridization probes. Restriction endonucleases: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sal*I. *Pst*I and *Bgl*II sites are incomplete. (A) ColV-K30. Solid bar indicates the regions in which IS*1* sequences map. Locations of aerobactin synthesis genes and 74K aerobactin receptor are indicated (4, 18). (B) The 16.3-kb *Hin*dIII fragment of ColV DNA in pABN1. This insert contains both aerobactin biosynthesis and transport genes (4). (C) *Hin*dIII-*Bgl*II subclone of pABN1 containing aerobactin biosynthesis genes. (D) *Hin*dIII fragment of pKLS1, a deletion mutant of pABN1. Dotted line indicates deleted sequences. (E) A 6.4-kb *Bam*HI fragment of pABN1 containing one copy of IS*1*.

iron medium, indicating expression of the gene(s) for the aerobactin receptor. The strains of *S. boydii* tested were found to synthesize and transport aerobactin. Neither synthesis nor utilization of enterobactin occurred in these strains.

To determine the presence of aerobactin genes directly, chromosomal and plasmid DNA isolated from strains of all four species was hybridized with the aerobactin genes cloned from the *E. coli* ColV plasmid (4). The 16.3-kb *Hin*dIII fragment of plasmid pABN1, which contains the aerobactin biosynthesis and transport genes (4) (Fig. 1B), was labeled by nick translation and hybridized to Southern blots of restriction enzyme-digested *Shigella* DNA. A large number of DNA fragments of *S. dysenteriae*, *S. sonnei*, and *S. flexneri* DNA, ranging in size from 0.9 to 23 kb, hybridized to the ColV sequences (Fig. 2, lanes B through D). The *S. flexneri* fragments could be seen more clearly when the filter was exposed for a longer period of time (data not shown), which overexposed the *S. dysenteriae* and *S. sonnei* fragments. The difference in intensity of *S. flexneri* fragments and those of *S. dysenteriae* and *S. sonnei* may reflect differences in copy number or in homology. Fewer fragments of *S. boydii* DNA were detected (Fig. 2A). Since the ColV probe was relatively large, it was necessary to use fragments or subclones of pABN1 (Fig. 1C and E) to determine which of the multiple bands represented aerobactin genes and whether the remaining bands represented a repeated sequence. One subclone, pKLS10, contained a 4.8-kb *Hin*dIII-*Bgl*II fragment of pABN1 (Fig. 1C). Iron transport mutants of *S. flexneri* were transformed with this plasmid to determine whether it contained aerobactin genes. SA201, a mutant defective in synthesis of aerobactin, was complemented by this plasmid, and aerobactin was detected in supernatant fluids of the transformant. SA255, a mutant defective in both synthesis and transport of aerobactin, was not complemented by pKLS10. Hybridization to this plasmid was used to detect aerobactin biosynthesis genes. When the *Hin*dIII-*Bgl*II fragment of this plasmid was used, a single *Hin*dIII (Fig. 2, lanes E, F, and H) or *Eco*RI (data not shown) fragment of *S. flexneri*, *S. sonnei*, or *S. boydii* hybridized under stringent conditions. The *Hin*dIII fragment was approximately 12.4 kb in *S. flexneri* and *S. sonnei* and approximately 11.0 kb in *S. boydii*. No hybridization to *S. dysenteriae* was detected (Fig. 2, lane G), even when less stringent conditions were used (data not shown).

Since aerobactin genes previously had been found to be plasmid encoded (29), *Shigella* plasmids were also hybridized to ColV aerobactin genes. Cleared lysates (10) of all four species contained one or more plasmids ranging in size from 1.5 to 240 kb, but none hybridized to the *Hin*dIII-*Bgl*II fragment of pKLS10 (Fig. 3).

The multiple bands detected when the entire *Hin*dIII fragment of pABN1 was used were also seen when a 6.4-kb *Bam*HI fragment of pABN1 (Fig. 1E) was hybridized to *Shigella* chromosomal DNA (Fig. 2, lanes I through L). This *Bam*HI probe shares 1.0 kb with pKLS10 and includes an additional 5.4 kb as determined by restriction enzyme digestion and hybridization.

The large number of fragments hybridizing to the *Bam*HI probe suggested that an insertion sequence might be present. Since a large number of copies (>40) of IS*1* are found in most *Shigella* species (20), it appeared likely that the sequence might be IS*1*. A probe containing IS*1* was used to determine whether this insertion sequence was present on pABN1. VAλ3, a λ phage which acquired a copy of IS*1* from R100 (7), was used as the source of IS*1* DNA. The 10.2-kb *Hin*dIII fragment of the phage containing IS*1* was hybridized to pABN1, and a single copy of IS*1* was found on the 6.4-kb *Bam*HI fragment (Fig. 4, lane C). No hybridization between wild-type λ and pABN1 was seen (data not shown), indicating that the homology between pABN1 and VAλ3 was due to IS*1* and not to the λ sequences. Digestion of pABN1 with *Pst*I produced two fragments which hybridized to IS*1* (Fig. 4, lane A), as was expected since there is a single *Pst*I site within IS*1* (21).

To determine whether additional copies of IS*1* were

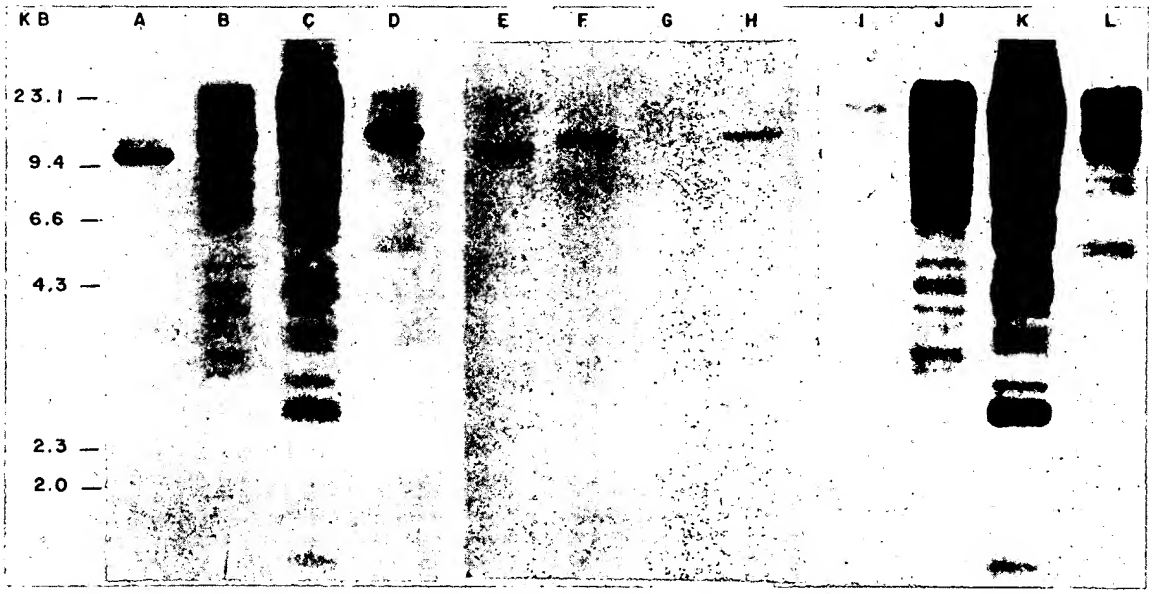

FIG. 2. Hybridization of ColV sequences to *Shigella* DNA. DNA of *S. boydii* (lanes A, E, and I), *S. sonnei* (lanes B, F, and J), and *S. dysenteriae* (lanes C, G, and K), and *S. flexneri* (lanes D, H, and L) was cut with *Hin*dIII and hybridized to the 16.3-kb *Hin*dIII fragment of pABN1 (lanes A through D), the 4.8-kb *Hin*dIII-*Bgl*II fragment of pKLS10 (lanes E through H), or the 6.4-kb *Bam*HI fragment of pABN1 (lanes I through L). Positions of molecular size standards are indicated at the left.

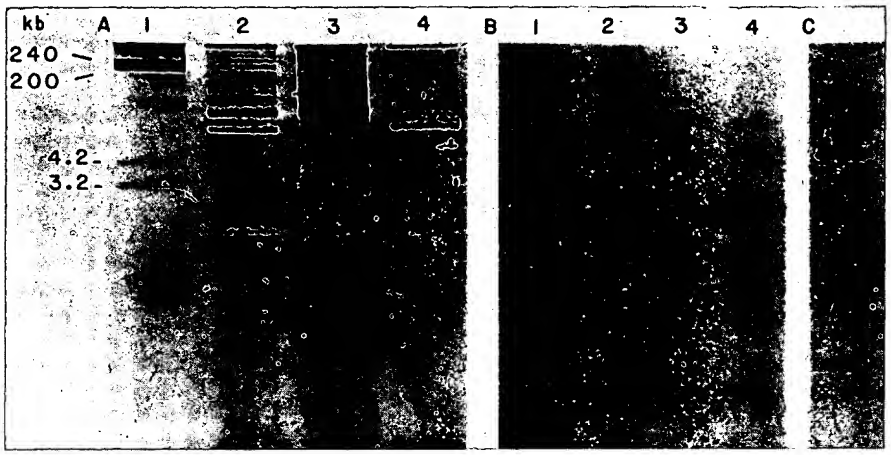

FIG. 3. Hybridization of ColV sequences to *Shigella* plasmids. (A) Plasmids of *S. flexneri* (lane 1), *S. sonnei* (lane 2), *S. boydii* (lane 3), and *S. dysenteriae* (lane 4) were electrophoresed in 0.9% agarose and were stained with ethidium bromide. A Southern blot of this gel (B) was hybridized to the 4.8-kb insert of pKLS10; lane numbers are as in (A). A sample of *S. flexneri* chromosomal DNA cut with *Hin*dIII (C) was included on the gel to ensure that hybridization would be detected. Sizes of *S. flexneri* plasmids are indicated at the left.

associated with the aerobactin genes, restriction enzyme fragments of the entire ColV plasmid were hybridized to pKLS10 (Fig. 5, lanes B and E) and VAλ3 (Fig. 5, lane G). A single 8.4-kb *Eco*RI fragment of ColV (Fig. 5, lane E) and a single 16.3-kb *Hin*dIII fragment (data not shown) were detected by using the aerobactin gene probe pKLS10. Two fragments were detected when the plasmid was cut with *Sal*I (Fig. 5, lane B) or *Bam*HI (data not shown), both of which cut within this region. The intensity of hybridization of the two *Sal*I fragments of ColV DNA (Fig. 5B) differs because *Sal*I cuts very near the end of the region detected by pKLS10 (Fig. 1), leaving only a small region of homology between pKLS10 and the 21-kb *Sal*I fragment.

A duplicate Southern blot was hybridized with the IS*1* probe to determine whether the ColV DNA fragments which hybridized to the aerobactin gene probe also contained IS*1* (Fig. 5). Four of the *Sal*I fragments (Fig. 5, lane G) and three of the *Eco*RI fragments (data not shown) hybridized to the IS*1* probe. Since these enzymes do not cut within IS*1* (21), there are at least four copies of the insertion sequence on the ColV plasmid. The different intensities of the four bands may indicate more than one copy of IS*1* on some fragments, or there may be differences in the extent of homology between ColV insertion sequences and the probe. Two copies of the insertion sequence are on 21- and 12.5-kb *Sal*I fragments, which also hybridize to the aerobactin gene probe, pKLS10 (Fig. 5, lanes B and G). One of these copies of IS*1* is contained on pABN1 (Fig. 4) but is absent from pKLS1 (Fig. 5, lane H), a deletion mutant of pABN1 which lacks the 12.5-kb *Sal*I fragment (Fig. 1D). This copy of IS*1* has been mapped 3′ to the aerobactin biosynthesis genes by enzyme digestion and hybridization (Fig. 4). A second copy lies 5′ to the aerobactin genes on the 21-kb *Sal*I fragment. This copy is to the left of the *Eco*RI site at position 20 kb on the ColV map (Fig. 1) since the 8.4-kb *Eco*RI fragment of ColV DNA which hybridized to pKLS10 did not hybridize to IS*1* (data not shown).

Hybridization of the ColV aerobactin genes (pKLS10) to *S. flexneri* and *S. boydii* DNA (Fig. 5) indicate considerable homology. *Eco*RI fragments of the same size, 8.4 kb, were detected in all three species under stringent conditions (Fig. 5, lanes D, E, and F). The flanking sequences are different, however, and the sizes of *Sal*I fragments (Fig 5, lanes A, B, and C) and *Bam*HI fragments (data not shown) differ in these species.

Hybridization of IS*1* to fragments of *S. flexneri* and *S. boydii* (data not shown) produced the same pattern obtained with the 6.4-kb *Bam*HI fragment of pABN1 (Fig. 2, lanes L and I). However, the large number of fragments in the chromosomal digests and the numerous copies of IS*1* in *S. flexneri* made it impossible to determine whether fragments of the same size which hybridize to both the IS*1* and aerobactin gene probes were in fact the same fragment.

If IS*1* is associated with the aerobactin genes in the

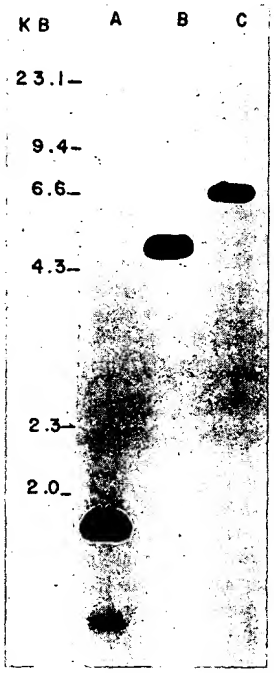

FIG. 4. IS*1* sequences of pABN1. pABN1 was cut with *Pst*I (lane A), *BGl*II (lane B), or *Bam*HI (lane C) and hybridized to the 10.2-kb *Hin*dIII fragment of VAλ3 containing IS*1*. Positions of molecular size standards are indicated at the left.

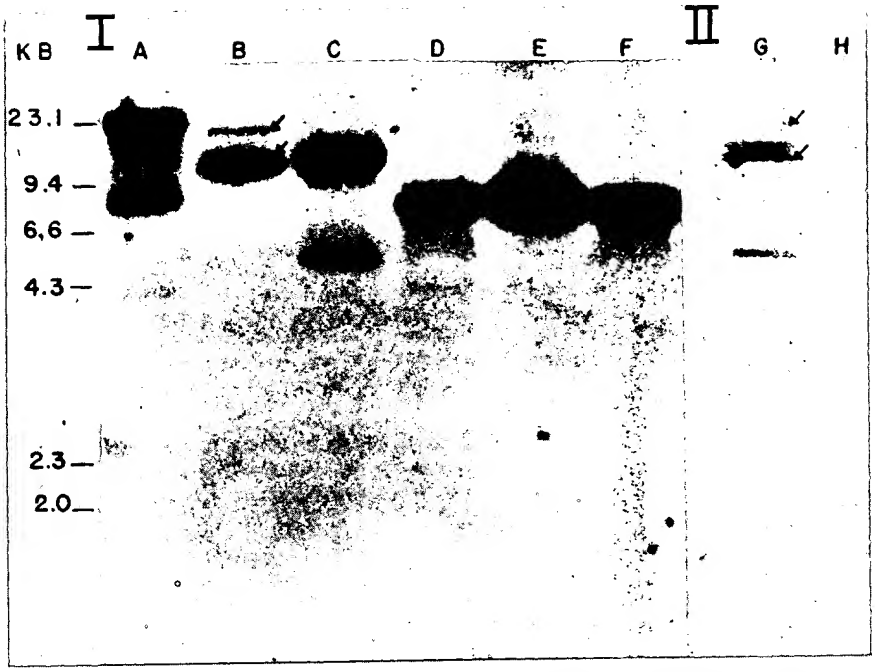

FIG. 5. Hybridization of aerobactin sequences to ColV, *S. flexneri*, and *S. boydii* and hybridization of IS*1* to ColV. DNA of *S. flexneri* (lanes A and D), *S. boydii* (lanes C and F), and ColV-K30 (lanes B and E) was cut with *Sal*I (lanes A through C) or *Eco*RI (lanes D through F) and hybridized to the 4.8-kb *Hin*dIII-*Bgl*II insert of pKLS10 containing aerobactin sequences. In addition, ColV-K30 (lane G) and pKLS1 (lane H) DNA were cut with *Sal*I and hybridized to the 10.2-kb *Hin*dIII fragment of VAλ3 containing IS*1*. Arrows indicate fragments which hybridized to both the aerobactin and IS*1* probes. Positions of standards, indicated at left, were the same for both gels.

Shigella chromosome as it is with the ColV aerobactin genes, a relatively high frequency of deletion mutations might be expected to occur in this region (5). Thirty-three independent, spontaneous mutants which failed to grow in low-iron medium were isolated from *S. flexneri* SA100 and were tested for synthesis and utilization of aerobactin. Twenty-six of these mutants failed to synthesize detectable aerobactin but were able to transport the compound; six mutants neither synthesized nor transported aerobactin. An additional mutant, SA280, was found to be defective in transport, but not in synthesis of the siderophore. This mutant reverted to wild type at a frequency of approximately 10^{-6}. None of the synthesis or synthesis and transport mutants reverted at a detectable frequency. This suggests that the majority of these mutants are deletions. DNA was isolated from two of the mutants, SA201 (a synthesis mutant) and SA255 (a synthesis and transport mutant), and was hybridized to pKLS10 (Fig. 6). SA201 DNA hybridized to pKLS10, but the size of the *Hin*dIII fragment was altered and the intensity was diminished compared with parental DNA (Fig. 6, lanes A and B). SA255 has no homology with pKLS10 (Fig. 6, lane C), indicating a deletion of aerobactin sequences. If the deletion of aerobactin genes in SA255 was due to IS*1*, the deletion should extend to the insertion sequence and alter the size of any restriction fragment containing IS*1* and flanking sequences. An IS*1* adjacent to the aerobactin sequences would not necessarily be on the same restriction fragment as the aerobactin genes. DNA from SA100 and SA255 was cut with *Hin*dIII, which does not cut within IS*1*, and hybridized to VAλ3. Two restriction fragments of 2.5 and 3.8 kb which were present in SA100 were absent from SA255 (Fig. 7). This indicates an alteration in sequences adjacent to the insertion sequence or deletion of the insertion sequence. The gel was exposed for a shorter period of time to determine changes in hybridization to larger fragments, but no differences were seen. Deletion of aerobactin genes as well as sequences adjacent to two copies of IS*1* suggests that the aerobactin genes of *S. flexneri*, like ColV, are flanked by copies of IS*1*. Deletions extending from one copy of IS*1* into the aerobactin genes or recombination between two copies of IS*1* would result in mutations of the type found in SA255. Smaller deletions would produce mutants defective in biosynthesis only.

Conjugation between an aerobactin-producing (Hds^+

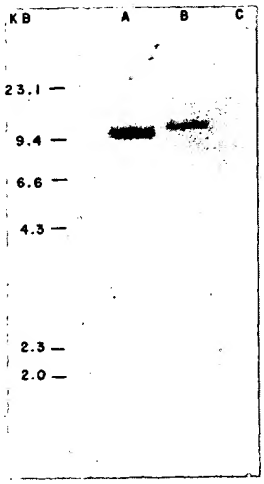

FIG. 6. Hybridization of aerobactin genes to *S. flexneri* mutants. DNA of *S. flexneri* SA100 (lane A), SA201 (lane B), and SA255 (lane C) was cut with *Hin*dIII and hybridized to the insert of pKLS10. Sizes of standards are indicated at the left.

FIG. 7. Hybridization of IS*1* to *S. flexneri*. DNA of SA100 (lane A) and SA255, an aerobactin biosynthesis and transport mutant of SA100 (lane B), was cut with *Hin*dIII and hybridized to the 10.2-kb *Hin*dIII fragment of VAλ3 containing IS*1*. Arrows indicate bands which are absent from SA255 DNA.

Hdu^+) donor and a streptomycin-resistant derivative of the deletion mutant SA255 was used to confirm the chromosomal location of these genes. The donor, strain 256, transfers the chromosome in a clockwise direction from approximately 7 min by analogy with the *E. coli* map (8). Hds^+ Hdu^+ recombinants were obtained at frequencies of 10^{-4} to 10^{-5}. This frequency was higher than that observed for other markers (10^{-5} to 10^{-8}), suggesting that the genes map near the origin of transfer. Linkage to other markers in this area, however, was not consistent. Additional studies will be done to determine the location of these genes.

These experiments were repeated with an F donor, SA845, instead of the Hfr donor. No Hds^+ Hdu^+ recombinants were obtained from these matings. Transfer by an Hfr but not an F donor indicates that the genes are chromosomal.

DISCUSSION

S. flexneri utilizes a hydroxamate compound, aerobactin, for transport of iron. This siderophore also has been isolated from other enteric bacteria. Although all strains of *S. flexneri* appear to use this compound, the aerobactin system is not uniformly present in other species. The somewhat erratic appearance of the genes within these species and their presence on a plasmid in some enteric bacteria suggests horizontal gene transmission.

Within the *Shigella* species, the genes for aerobactin synthesis are chromosomal and are closely linked. Hybridization studies indicate the genes are contained on a single *Eco*RI restriction endonuclease fragment of *S. flexneri*, *S. boydii*, and *S. sonnei* DNA. These sequences show considerable homology with the ColV aerobactin sequences as they hybridize under stringent conditions. Although *S. sonnei* contains the genes for aerobactin synthesis as indicated by hybridization to the ColV aerobactin sequences, no detectable aerobactin was produced. Members of this species synthesize enterobactin and thus have the genetic information for two separate iron transport systems. The presence of a functional phenolate iron transport system may have permitted the accumulation of mutations in the aerobactin biosynthetic genes of *S. sonnei*. The genes encoding the aerobactin receptor and aerobactin utilization in *S. sonnei* have remained intact since *S. sonnei* is able to transport and utilize exogenous aerobactin. Although *S. sonnei* has been reported to synthesize a hydroxamate distinct from aerobactin and one strain of *S. boydii* was reported to make a phenolate siderophore (25), this was not observed in any of the strains we tested.

Unlike the other *Shigella* species, *S. dysenteriae* lacks the genes for the aerobactin iron transport system. Chemical and bioassays were negative for this compound, and sequences homologous to the ColV aerobactin genes were not detected. Since the aerobactin genes are found in all the other *Shigella* species, it is possible that the genes were present at one time in *S. dysenteriae* but were lost by IS*1*-mediated deletion in the strains we assayed.

Restriction enzyme digestion and hybridization have demonstrated the presence of the insertion sequence IS*1* flanking the ColV aerobactin genes. Studies by McDougall and Neilands (18) and Perez-Casal and Crosa (24) have also shown that the ColV aerobactin genes are flanked by IS*1*.

The large number of copies of IS*1* in the *S. flexneri* chromosome makes it difficult to determine by hybridization whether any of the IS*1* copies are closely linked to the aerobactin genes. However, the high frequency of deletion mutants, and the changes in hybridization to both aerobactin genes and IS*1* in SA255, suggests that one or more copies of IS*1* are in close proximity to these genes.

The presence of these insertion sequences could promote transposition as well as deletion of the aerobactin genes (5, 11). In addition, IS*1* could provide a site for recombination between DNA containing the aerobactin genes and other DNA elements which have IS*1* (11). Since the aerobactin genes have been associated with increased virulence in *E. coli* strains (29), the spread of these genes through transposition or recombination is of potential medical significance.

ACKNOWLEDGMENTS

We are indebted to J. B. Neilands, A. Bindereif, and S. McDougall for providing the cloned aerobactin genes and for helpful discussions and to S. McIntire for providing the lambda carrying IS*1*. We thank J. Stoebner High for expert technical assistance.

This study was supported by grant F-941 from the Robert A. Welch Foundation.

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